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(54) Title: SCREEN FOR RISK FOR GASTRIC ADENOCARCINOMA

(57) Abstract

It has been determined that a specific metaplastic lineage that contains immunoreactivity for a trefoil polypeptide, spasmolytic peptide, is associated with and gives rise to the vast majority of human adenocarcinomas. The identification of this Spasmolytic Polypeptide Expressing Metaplasia (SPEM) is a major factor for grading of biopsies of the stomach to assess risk for gastric cancer. It also forms the basis of a method for serological screening for those at risk for gastric cancer. In a preferred embodiment, antibodies to spasmolytic peptide (hSP) are used in immunostaining of biopsies of gastric tissue obtained by endoscopy for grading biopsies. Those patients having these cells, characterized by a morphology more typical of a type of cell present normally in the intestine and not stomach, Brunner's gland cells, are at risk of developing adenocarcinoma. Since these cells express hSP, antibodies or nucleic acid probes hybridizing to mRNA encoding hSP, can be used for rapid detection of the cells in tissue biopsies. The antibodies can also be used in serological tests for screening and following patients at risk for gastric cancer. In combination with evidence of previous or present infection with *H. pylori*, the test are predictive of the likelihood of developing adenocarcinoma.

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SCREEN FOR RISK FOR GASTRIC ADENOCARCINOMA

Background of the Invention

The United States government has certain rights in this invention by virtue of a grant from the National Institutes of Health NIDDKD to James R. Goldenring.

Gastric cancer worldwide is the second highest cause of death from cancer. While rates of gastric cancer have been decreasing in the United States, gastric cancer prevalence remains high in other parts of the world, especially in Asia. Presently, patients in endemic regions for gastric center, especially Japan and China, undergo screening by upper endoscopy. There is no alternative at present for discovery of early cancer other than by endoscopy. Grading of these endoscopies is based solely on hematoxylin and eosin staining. There are no prognostic markers that could indicate those at risk for future cancer development. Therefore, while biopsies can reveal early gastric cancers, patients in high risk regions who are initially negative by endoscopy must be followed with endoscopies to rule out future developments.

The epidemiological association between chronic *H. pylori* infection and gastric carcinoma is now well established, such that the Working Group Meeting of the International Agency for Research on Cancer with the World Health Organization recently classified *Helicobacter pylori* as a group 1 human gastric carcinogen (Peura, D.A. *Gatroenterology*. **113**, S4-S8 (1997)). Parsonnet and colleagues reported that 84% of patients with gastric cancer had serum antibodies against *H. pylori*, as opposed to 66% of uninfected matched controls (Parsonnet, et al. *New Eng. J. Med.* **325**, 1127-1131 (1991)). *H. pylori* infection induces a chronic gastritis progressing to atrophic gastritis, foveolar hyperplasia and intestinal metaplasia (Mobley, H.T.L. *Gastronenterology*. **113**, S21-S28 (1997)). Recent studies have indicated that chronic gastritis associated with *Helicobacter pylori* contributes to the development of

gastric adenocarcinoma (Peura, D.A. *Gatroenterology.* **113**, S4-S8 (1997); Parsonnet, et al. *New Eng. J. Med.* **325**, 1127-1131 (1991); Forman et al. *Br. J. Med.* **302**, 1302-1305 (1991); Nomura, et al. *New Eng. J. Med.* **325**, 1132-1136 (1991)). Nevertheless, the mechanism of progression from chronic gastritis to malignant disease remains unclear, and the relationship of intestinal metaplasia, *H. pylori* infection and the development of cancer continues to be controversial. Moreover, while an association between gastric cancer and infection with *H. pylori* has recently been established, the cell of origin for gastric adenocarcinoma is controversial. This does not establish a mechanism between the bacteria and the cancer, and provides little or no guidance for correlating treatment of, or risk associated with, *H. pylori* as it relates to development of gastric cancer.

It is therefore an object of the present invention to provide screening methods for early gastric cancer.

It is a further object of the present invention to provide means for assessing the degree of early gastric cancer and for screening and following patients at risk for gastric cancer.

It is a still further object of the present invention to provide means for serological testing for patients at risk of gastric cancer.

Summary of the Invention

It has been determined that a specific metaplastic lineage that contains immunoreactivity for a trefoil polypeptide, spasmolytic peptide, is associated with and gives rise to the vast majority of human adenocarcinomas. The identification of this Spasmolytic Polypeptide Expressing Metaplasia (SPEM) is a major factor for grading of biopsies of the stomach to assess risk for gastric cancer. It also forms the basis of a method for serological screening for those at risk for gastric cancer. In a preferred embodiment, antibodies to spasmolytic peptide (hSP) are used in immunostaining of biopsies of gastric tissue obtained by endoscopy for grading biopsies. Those patients having these cells, characterized by a

morphology more typical of a type of cell present normally in the intestine and not stomach, Brunner's gland cells, are at risk of developing adenocarinoma. Since these cells express hSP, antibodies or nucleic acid probes hybridizing to mRNA encoding hSP, can be used for rapid detection of the cells in tissue biopsies. The antibodies can also be used in serological tests for screening and following patients at risk for gastric cancer. In combination with evidence of previous or present invention with *H. pylori*, the tests are predictive of the liklihood of developing adenocarcinoma.

Detailed Description of the Invention

Metaplastic cell lineages arising in response to chronic injury are precursors for the evolution of dysplasia and adenocarcinoma. This sequence is well characterized in the case of the Barrett's epithelium, a columnar specialized intestinal metaplasia in the distal esophagus of patients with esophageal reflux (Haggitt, R.C. *Hum. Pathol.* **25**, 982-993 (1994)). While a subtype of intestinal metaplasia has been associated with gastric adenocarcinoma (Dixon, et al. *Am. J. Surg. Pathol.* **20**, 1161-1181 (1996); Filipe et al. *Int. J. Cancer.* **57**, 324-329 (1994)); Correa, P. *Cancer Res.* **48**, 3554-3560 (1988)), the link between these lineages and the evolution of gastric adenocarcinoma has not been clear. It has now been determined that there is an association between SPEM, detectable in biopsies based on the presence of cells having a morphology similar to Brunner's gland cells, and adenocarcinoma. Although normal cells in the stomach, such as mucus neck cells, express hSP, these cells are not predictive of adenocarcinoma.

I. Methods and Reagents for Screening

A. Histology of the Gastric Tissues

Presently, gastric mucosal biopsies are fixed in formalin and embedded in paraffin. Microtome sections of tissues are then stained with hematoxylin and eosin. These stained slides are examined for loss of parietal cells (oxyntic atrophy), ulceration, inflammatory infiltrates, and

alterations in cell lineages including increased numbers of surface mucous cells (foveolar hyperplasia), the presence of goblet cells (intestinal metaplasia), as well as dysplasia and adenocarcinoma. Dysplasia and adenocarcinoma ar judged by changes in nuclear morphology, loss of cytoplasmic space, loss of polarity and invasion of submucosa or vasculature. Brunner's glansds are not present in the normal stomach but can be observed in the duodenum.

B. Markers of SPEM

Small peptides displaying a cysteine-rich module (termed P-domain or trefoil motif) from a group of peptides, including BCEI, expressed from the pS2 gene; hITF, expressed from the TFF3 gene; and hSP, expressed from the SML1 gene. These peptides are abundantly expressed at mucosal surfaces of specific tissues and are associated with the maintenance of surface integrity. (Schmitt, et al., *Cytogenet. Cell Genet.* **72(4)**, 299-302 (1996)). Human spasmolytic peptide (hSP) was identified by Romasetto, et al., *EMBO J.*, **9(2)**, 407-414 (1990), based on homology to pancreatic spasmolytic polypeptide, sequenced and determined to be separately encoded on the genome from pS2. The gene sequence and amino acid sequences of hSP can be obtained from GenBank, accession number 1477545. Both are present in normal stomach epithelium. The patterns and timing of the expression of the trefoil peptides are different from each other. It is though that S2 plays an important rle in the proliferation of intestinal epithelial cells during the acute phase of mucosal ulceration, whereas ITF may be involved in differentialtion of the cells, particularly to form goblet cells, during the recovery phase of acute colitis. (Itoh, et al., *Biochem. J.* **318(Pt 3)**, 939-944 (1996)). Immunostaining for SP in the intact mucosa has been determined to be confined to the mucous neck cells, but following exposure to stress it was significantly enhanced and occurred also in the cells of the basal region of gastric glands, as reported by Konturek, et al., *Regul. Pept.* **68(1)**, 71-79 (1997). Konturek, et al. (1997) proposed that SP plays an important role in healing of stress-induced gastric lesions, possibly by the acceleration of

the mucosal repair, the enhancement of mucosal blood flow and the inhibition of gastric secretion.

It has now been determined that SP is a marker of metaplastic cells having a morphology similar to those of Brunner's gland cells. These cells can be identified by histological examination. However, the identification of SPEM with spasmolytic peptide immunostaining is easier, more sensitive and rapid. Therefore, detection of metaplastic cells expressing SP provides a means for identification of those at risk who would need further follow-up. Furthermore, since the SPEM lineage is often extensive, quantitation of serum spasmolytic polypeptide levels by either radioimmunoassay or ELISA should be useful to stratify patients at risk and provide a serological method for identifying and following patients at risk for developing adenocarcinoma.

SPEM can be detected using antibodies or antibody fragments prepared by standard techniques. The studies described in the examples were performed with a mouse monoclonal IgM anti-human spasmolytic polypeptide developed by Drs. Richard Poulsom and Nicholas Wright at the Imperial Cancer Research Fund, London, UK. Antibodies specifically directed towards utility in RIA and ELISA have also been developed. Either monoclonal or polyclonal antibodies can be used. Antibodies can be labelled using any detectable marker, including radiolabels, fluorescent labels, dyes, enzyme-chromogenic substrate systems, and other means commercially available.

SPEM can also be detected using nucleic acid probes which hybridize under standard hybridization conditions, as described for example, by Maniatis, et al. Molecular Cloning: A Laboratory Manual (Cold Spring Harbor Laboratory), to mRNA encoding SP. These can be labeled using standard labelling techniques for detection of bound nucleic acid.

Alternatively, or in addition, other markers of these cells can be used to screen for the presence of SPEM in gastric tissue biopsies.

For serological assay of SP, serum would be obtained from fasting patients. SP levels would be determined using either radioimmunoassay or enzyme-linked immunoassay. A standard curve would be used for known amounts of recombinant SP (Lars Thimm, Novartis Corporation). Patients with elevated levels of SP in serum would be investigated for the presence of SPEM by endoscopy. Alternatively, patients with elevated serum SP and documented SPEM could be followed following treatment of *H. pylori* by serial serum determinations of SP.

C. Detection of *H. pylori* Infection

Since *H. pylori* is known to be associated with an increased incidence of adenocarcinoma of the stomach, efficacy of screening can be further enhanced by testing for previous or present *H. pylori* infection. *H. pylori* infestion would be determined by either CLO test at the time of biopsy or *H. pylori* serology with the same sample used for SP determination.

II. Patients to be Screened and Screening Procedures

SP detection, such as immunohistochemistry, can be used to determine the presence of SPEM in endoscopic biopsies as well as brushings obtained either through endoscopy or blind per oral intubation.

As noted above, gastric cancer screening, especially in Asian countries, is a major focus for clinical care. Presently, the only impact of medicine on gastric cancer is through early detection of low grade tumors and early resection for cure. High grade tumors have uniformly dismal prognosis with median survival less than one year. No significant effect of adjuvant chemotherapy has been noted. The addition of spasmolytic polypeptide immunostaining of biopsies and the identification of SEEM provides a means for identifying those at risk for developing cancer in the future. Similarly, the use of spasmolytic peptide serology should provide a blood test for identification of those at risk. Thus, the use of spasmolytic polypeptide immunostaining could significantly decrease the number of screening endoscopies, focus screening endoscopies, and,

through serology testing, facilitate screening of large populations a risk in Asia and other countries with high cancer incidence.

The present invention will be further understood by reference to the following non-limiting examples.

Background

Wang and colleagues examined the influences of chronic *Helicobacter* gastritis using *Helicobacter felis* to infect the gastric epithelium of C57BL/6 mice (Wang, et al. *Gastroenterology*. **114**, 675-689 (1998)). Infection with *H. felis* produced a chronic gastritis with pathologic features similar to human infection with *H. pylori* including marked loss of parietal and chief cell populations, in addition to elaboration of an aberrant mucous cell lineage (Lee, et al. *Gastroenterology*. **99**, 1315-1323 (1990); Fox, et al., *Gastroenterology*. **110**, 155-166 (1996)). Wang, et al. (1998) reported that an aberrant metaplastic cell lineage with morphological characteristics similar to runner's glands of the duodenum develops in the fundic mucosa of mice infected with *H. felis*. This metaplastic lineage expresses the trefoil peptide spasmolytic polypeptide (SP). This expanded lineage stained with antibodies against spasmolytic polypeptide (SP), a trefoil peptide secreted from mucous neck cells in the normal fundic mucosa (Wang, et al. (1998); Thim, L. FEBS Lett. 250, 85-90 (1989)). Importantly, the *H. felis*-induced SP-expressing metaplastic (SPEM) lineage did not show morphological characteristics of mucous neck cells, but rather demonstrated morphology more reminiscent of Brunner's glands of the duodenum (Wang (1998)).

Hypothesis

Given the results in mice, studies were designed to investigate whether *H. pylori* infection would induce a similar aberrant SP-expressing lineage in human fundic mucosa. Given the epidemiological association of *Helicobacter sp.* infection with gastric cancer, it was hypothesized that this SP-expressing metaplastic (SPEM) lineage may represent a precursor to or appear commensurate with gastric adenocarcinoma.

Summary of Results

The results of these studies showed that the SPEM lineage was present in 68% of fundic biopsies from patients with fundic *H. pylori*-associated gastritis, but was absent in biopsies of fundic mucosa from patients without *H. pylori* infection. In a review of archival samples from 22 resected gastric adenocarcinomas, the SPEM lineage was found in 91% of cases, typically located in mucosa adjacent to the carcinoma or areas of dysplasia. Importantly, 56% of samples showed SP immunoreactivity within dysplastic cells. These data indicate not only a strong association between the SPEM lineage and gastric adenocarcinoma, but that SPEM cells represent the metaplastic lineage responsible for development of this tumor in patients with *H. pylori*.

Example 1: Detection of SP levels in gastric fundic biopsies.

Ten fundic biopsies from *H. pylori* negative patients and 17 biopsies from patients with *H. pylori* colonization in the fundus were examined. All biopsies demonstrated H/K-ATPase-immunostaining parietal cells and contained no gastrin-immunoreactive cells, confirming the authenticity of fundic mucosal biopsies. In all of the *H. pylori*-infected biopsies specimens, the presence of *H. pylori* was confirmed by Giemsa staining of tissue sections. The histological characteristics of these biopsies are summarized in Table I.

Table I: Histological analysis of fundic biopsies from *H. pylori* (Hp) negative and *H. pylori* positive patients.

	HP Negative		HP Positive	
	Absent	Present	Absent	Present
Foveolar hyperplasia	10	0	4	13
Oxyntic atrophy	10	0	6	11
Mononuclear infiltrates	8	2	1	16*
SPEM	10	0	6	11

Foveolar hyperplasia was assessed in sections stained for pS2 as the presence of surface cells in greater than 25% of the gland length. Oxyntic atrophy was assessed in sections stained for H/K-ATPase as a decrease in parietal cell density to less than half the gland length. Mononuclear infiltrates were assessed in H&E stains.

*3 of 16 *H. pylori* (Hp) positive patients demonstrated organized lymphoid aggregates. SPEM was assessed in sections stained for hSP.

Gastric fundic biopsies were stained for SP. 10 μm sections from paraffin-embedded biopsies of fundic mucosa from *H. pylori* uninfected and *H. pylori* infected patients were stained for hSP with a monoclonal murine anti-HSP Elia, et al. *Histochem.* J. **26**, 644-647 (1994)). Dewaxed paraffin sections were blocked with 5% goat serum in phosphate buffered saline and then incubated with anti-hSP (1:50) for one hour at room temperature. Indirect immunohistochemical detection was then performed through incubation with biotinylated anti-mouse IgG, streptavidin-conjugated alkaline phosphatase and finally Vector Red chromogen substrate (Vector Laboratories, Burlingame, CA). For confirmation of the veracity of biopsies as fundic mucosa, serial sections were also stained with monoclonal antibodies against the H/K-ATPase

(1:5000, the gift of Dr. Adam Smolka, Medical University of South Carolina, a marker of parietal cells) and gastrin (undiluted, Zymed, a marker of antral G-cells).

The presence of *H. pylori* was confirmed in all biopsies with Giemsa staining. All biopsies demonstrated immunoreactive parietal cells without the presence of G-cells). In non-infected patients, staining was confined to normal appearing mucous neck cells. In *H pylori*-infected patients, nodular aggregates of hSP-staining cells with the characteristics of Brunner's gland cells (SPEM) were observed in 65% of biopsies. No SPEM was observed in fundic biopsies from non-infected patients.

In non-infected patients, fundic biopsies demonstrated only normal appearing SP-immunoreactive mucous neck cells. In contrast, 65% of biopsies from *H. pylori* infected biopsies exhibited aberrant hSP-immunoreactive SPEM cells. The lineage showed a morphology more characteristic of Brunner's gland cells. Most cells were present as nodular formations in the deeper portions of the metaplastic glands. Foveolar hyperplasia was present in 70% of *H. pylori* infected biopsies (Table I). Significant mononuclear infiltrate was present in 95% of biopsies with 18% of the biopsies demonstrating organized lymphoid aggregates. These results indicated that the SPEM lineage was present in the fundic mucosa of many patients with *H. pylori*-associated fundic gastritis.

Example 2: Association of SPEM with Gastric Adenocarcinomas.

Since chronic *H. pylori* infection is associated with the development of adenocarcinoma, archived tissues from resected gastric adenocarcinomas in patients were examined. All of the resections were for tumors of the fundus or fundal/antral border.

Ten μm sections of paraffin-embedded mucosa from regions adjacent to gross adenocarcinoma were examined for immunostaining with hSP antibodies as described above. Prominent regions of SPEM were observed in mucosa underlying regions of foveolar hyperplasia. The lineage was always observed near the bases of glands. As noted in the

biopsies, the SPEM cells were large with extensive numbers of immunoreactive granules. In an adjacent region, hSP staining of normal-appearing mucous neck cells was observed. Parietal cells were also observed in these sections. Adjacent sections were stained with anti-PSTI (1:200) with staining as described above except that sections underwent retrieval using citrate buffer in a microwave and horseradish peroxidase-conjugated secondary antibodies were used for detection with diaminobenzidine chromogen. While distinct PSTI immunoreactive mucous neck cells were observed within the mucosa, the SPEM lineage cells at the right side of the section showed no PSTI immunoreactivity. Adjacent sections were also stained with anti-PS2 (1:50) as described above. While pS2 immunostaining was observed in regions of surface cell foveolar hyperplasia, SPEM cells did not stain for pS2. Toluidine blue staining of a 0.5 μm section of glutaraldehyde fixed tissue from a resection specimen demonstrates the numerous granules in SPEM cells. Note the presence of residual parietal cells in some SPEM-containing glands, confirming the presence of the lineage in fundic mucosa. Electron microscopic examination demonstrates the presence of multiple mucous granules within the SPEM cells along with the characteristic expanded apical membrane surface without microvilli.

In summary, twenty patients (91%) demonstrated the SPEM lineage within their resection specimens. No evidence of the lineage was observed in two patients with diffuse infiltrating adenocarcinoma. In most specimens, a prominent homogeneous cell population expressing hSP was noted dominating greater than half the lower length of the fundic glands. These SPEM cells possessed an abundance of cytoplasmic mucin granules and a wide apical surface. Branching of SPEM-containing glands was observed in many specimens. Most glands displayed varying degrees of oxyntic cell atrophy, however there was more variability in expansion of the surface cell compartment, with 68% of patients having foveolar hyperplasia. Normal mucous neck cell staining with hSP and parietal cells were observed in regions adjacent to SPEM-containing

regions. Goblet cell-containing intestinal metaplasia was only observed in 10% of specimens.

Resection specimens were stained with several lineage-specific markers to characterize the origin of the SPEM lineage more completely. Increased expression of TGFα has been noted previously in foveolar cells of patients with Menetrier's disease and hypertrophic lymphocytic gastritis (Dempsey, et al. *Gastroenterology.* **103**, 1950-1963 (1992); Bluth, et al. *Hum. Pathol.* **26**, 1333-1340 (1995)). However, clear overexpression of TGFα in the foveolar regions of the mucosa adjacent to tumor was not observed. In addition, the SPEM lineage did not stain with antibodies against TGFα. In all cases, there was no evidence of enterochromaffin-like (ECL) cell hyperplasia, although scattered chromogranin A immunoreactive cells were observed. The SPEM lineage did not stain with antibodies against chromogranin A. While antibodies against pancreatic secretory trypsin inhibitor (PSTI) did stain scattered normal appearing mucous neck cells (McKenzie, et al. *Am. J. Physiol.* **273**, G112-G1117 (1997)), no staining of the lineage was observed. While antibodies against the trefoil peptide pS2 did stain surface mucous cells in areas of foveolar hyperplasia, no staining was observed in the SPEM lineage. Previous investigations have suggested that Intestinal Trefoil Factor (ITF), which is absent from the normal gastric mucosa, is unregulated following injury (Podolsky, et al. *J. Biol. Chem.* **268**, 6694-6702 (1993); Alison, et al. *J. Pathol.* **175**, 405-414 (1995)). While occasional cells expressing ITF mRNA could be identified in regions of intestinal metaplasia, no ITF expression could be documented in the SPEM lineage. Finally, examination of SPEM in both thick and thin plastic sections demonstrated the presence of abundant mucous granules and a broadened apical surface in these cells. These ultrastructural characteristics are similar to those of SPEM cells observed in H. felis-infected C57BL/6 mice (Wang (1998)). All of these findings indicate that the SPEM lineage is not directly derived from a normal gastric lineage. Rather it appears to represent a metaplastic lineage and is differentiable

from both normal mucous neck cells and the goblet cell-containing type metaplasia previously associated with chronic *H. pylori* infection and atrophic gastritis (Dixon, et al. (1996)).

As noted above, the SPEM lineage was observed in apposition with both dysplastic lineages as well as adenocarcinoma. hSP staining was observed in both SPEM as well as in contiguous regions of dysplasia in 49% of resection samples. Immunoreactivity was detectable in a region of adenocarcinoma adjacent to the hSP-immunoreactive cells. At higher magnification, dysplastic epithelial cells appear to be contiguous with SPEM cell-containing glands. Importantly, however, in 59% of the resections, hSP immunoreactivity was present in cells within regions of severe dysplasia or carcinoma *in situ*. In several cases regions of SP-expressing dysplastic cells were noted extending from regions of SPEM lineage. While regions containing the SPEM lineage did not show significant labelling with Ki-67 and PCNA antibodies, the dysplastic areas showed prominent nuclear labelling in addition to characteristic changes in cell morphology. These results indicate that the SPEM lineage may represent a metaplastic precursor for the development of dysplasia and adenocarcinoma.

Previous investigations have focused on the association of Type III intestinal metaplasia with the development of adenocarcinoma. Despite this association, it is less clear that neoplastic cells actually arise from areas in goblet-cell containing intestinal metaplasia. Of note, while intestinal metaplasia and foveolar hyperplasia are often conspicuous in atrophic gastritis in association with *H. pylori* infection, gastric adenocarcinomas usually develop deep in the glands as nodular lesions that underlie the regions of intestinal metaplasia or foveolar hyperplasia. The identification of the SPEM lineage supports the proposal that this metaplastic candidate precursor is located in proximity with the putative site of neoplastic transition.

It is not clear whether hSP itself might contribute to the development of adenocarcinoma. hSP has generally been associated with

cytoprotection in the gastric mucosa (McKenzie, et al., (1997); Tanaka, et al. *Am. J. Physiol.* **272**, G1473-G1780 (1997); Babyatsky, et al. *Gastroenterology.* **110**, 489-497 (1996)). Homologous deletion of the gene for the gastric surface cell trefoil protein pS2 leads to the development of intramucosal carcinomas (Lefebvre, et al. *Science* **274**, 259-262 (1996)), however the lineage responsible for these lesions is unclear. It is more likely that the expression of SP is a functional marker of this metaplastic lineage with Brunner's gland morphology. A metaplasia with Brunner's gland morphology expressing hSP has been associated with acute and chronic injury of the gastrointestinal mucosae (Wright, et al. *Nature.* **343**, 82-85 (1990); Ahnen, et al. *J. Pathol.* **173**, 317-326 (1994)). Nevertheless, the ulcer-associated cell lineage (UACL) appears to constitute a separate entity, since SPEM does not express ITF. Nevertheless, one can not rule out that SPEM might evolve from UACL in the gastric mucosa.

The presence of a well differentiated metaplastic lesion as a precursor of adenocarcinoma is not without precedence. Esophageal adenocarcinoma develops from dysplastic transition within the metaplastic columnar Barrett's epithelium in the distal esophagus (Reid, et al. *Gasronenterology.* **102**, 1212-1219 (1992); Miros, et al. *Gut.* **32**, 1441-1446 (1991)). As with the SPEM lineage in the stomach, the Barrett's epithelium shows low proliferative indices. Dysplasia and adenocarcinoma only develop in a minority (10-15%) of patients. These studies indicate that, while the SPEM lineage is commonly associated with fundic *H. pylori* gastritis, only a minority of patients will progress to dysplasia and adenocarcinoma. It is therefore likely that further events must occur to elicit the development of the neoplastic transition. Thus, as recognition and monitoring of Barrett's epithelium is the mainstay of surveillance for the development of esophageal adenocarcinoma (Robertson, et al, M. *Br. J. Surg.* **75**, 760-763 (1988)), so surveillance of patients at risk for gastric adenocarcinoma should focus attention on this metaplastic precursor lineage expressing SP.

In summary, these data support the hypothesis that the SPEM lineage is a link between chronic *H. pylori* gastritis and dysplasia leading to gastric adenocarcinoma.

We claim:

1. A method for screening for the liklihood of developing adenocarcinoma comprising detecting the presence of metaplastic cells expressing spasmolytic peptide which have Brunner's gland morphology in gastric tissues in a patient.

2. The method of claim 1 wherein the gastric tissues are detected using reagents specific for spasmolytic peptide.

3. The method of claim 2 wherein expression is detection by hybridization of a nucleic acid probe to mRNA encoding spasmolytic peptide.

4. The method of claim 2 wherein expression is detected by detection of spasmolytic peptide in the gastric tissues.

5. The method of claim 4 wherein the peptide is detected using labelled antibody to spasmolytic peptide.

6. The method of claim 1 wherein expression of the spasmolytic peptide is determined in a biopsy of gastric tissues obtained by endoscopy.

7. The method of claim 1 wherein the spasmolytic peptide is measured in a blood sample.

8. The method of claim 1 further comprising determining if the patient has been infected by *Helicobacter pylori*.

9. A screening kit for for the liklihood of developing adenocarcinoma comprising reagents for detecting the expression of spasmolytic peptide in gastric tissues in a patient at risk of adenocarcinoma.

10. The kit of claim 9 comprising labelled antibodies to spasmolytic peptide.

11. The kit of claim 10 wherein the antibodies are directly or indirectly labelled with a reagent selected from the group consisting of fluorescent labels, radiolabels, enzyme labels, and dyes.

12. The kit of claim 9 wherein the reagents are labelled nucleic acid probes hybridizing under standard conditions to mRNA encoding spasmolytic peptide.

13. The kit of claim 9 further comprising reagents for determining if the patient has been infected by *Helicobacter pylori*.

14. The kit of claim 9 further comprising materials for collecting gastric tissue samples.

15. The kit of claim 14 further comprising histological stains.

INTERNATIONAL SEARCH REPORT

Inter. .onal Application No
PCT/US 98/20820

A. CLASSIFICATION OF SUBJECT MATTER
IPC 6 G01N33/574 G01N33/68

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 6 G01N C07K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	SCHMIDT, P. H. (1) ET AL: "Association of an aberrant spasmolytic peptide -expressing cell lineage with gastric adenocarcinoma in humans." GASTROENTEROLOGY, (APRIL 15, 1998) VOL. 114, NO. 4 PART 2, PP. A673. MEETING INFO.: DIGESTIVE DISEASE WEEK AND THE 99TH ANNUAL MEETING OF THE AMERICAN GASTROENTEROLOGICAL ASSOCIATION NEW ORLEANS, LOUISIANA, USA MAY 16-22, 1998 AMERICAN GASTROENTEROLO, XP002107070 see abstract ---	1-15
A	WO 96 15456 A (LOCUS GENEX OY ;HAERKOENEN MATTI (FI)) 23 May 1996 see the whole document --- -/--	1,9

[X] Further documents are listed in the continuation of box C.

[X] Patent family members are listed in annex.

° Special categories of cited documents :

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Date of the actual completion of the international search	Date of mailing of the international search report
24 June 1999	12/07/1999

Name and mailing address of the ISA	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Tx. 31 651 epo nl, Fax: (+31-70) 340-3016	Moreno, C

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INTERNATIONAL SEARCH REPORT

Inter. .onal Application No
PCT/US 98/20820

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

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